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Experimental muscle hyperalgesia modulates sensorimotor cortical excitability, which is partially altered by unaccustomed exercise

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Abstract

Impaired corticomotor function is reported in patients with lateral epicondylalgia, but the causal link to pain or musculotendinous overloading is unclear. In this study, sensorimotor cortical changes were investigated using a model of persistent pain combined with an overloading condition. In 24 healthy subjects, the effect of nerve growth factor (NGF) induced pain, combined with delayed onset muscle soreness (DOMS), was examined on pain perception, pressure pain sensitivity, maximal force, and sensorimotor cortical excitability. Two groups (NGF alone, NGF+DOMS) received injections of NGF into the extensor carpi radialis brevis (ECRB) muscle at Day-0, Day-2, and Day-4. At Day-4, the NGF+DOMS group undertook wrist eccentric exercise to induce DOMS in the ECRB muscle. Muscle soreness scores, pressure pain thresholds (PPT) over the ECRB muscle, maximal grip force, transcranial magnetic stimulation (TMS) mapping of the cortical ECRB muscle representation and somatosensory evoked potentials (SEPs) from radial nerve stimulation were recorded at Day-0, Day-4 and Day-6. Compared with Day-0, Day-4 showed in both groups: 1) Increased muscle soreness ($P<0.01$); 2) Reduced PPTs ($P<0.01$); 3) Increased motor map volume ($P<0.01$); 4) Decreased frontal N30 SEP. At Day-6, compared with Day-4, only the DOMS+NGF group showed: 1) Increased muscle soreness score ($P<0.01$); 2) Decreased grip force ($P<0.01$); 3) Decreased motor map volume ($P<0.05$). The NGF group did not show any difference on the remaining outcomes from Day-4 to Day-6. These data suggest that sustained muscle pain modulates sensorimotor cortical excitability and that exercise-induced DOMS alters pain-related corticomotor adaptation.

Key words: Persistent muscle hyperalgesia, exercise-induced pain, neuroplasticity, cortical somatosensory excitability

INTRODUCTION

Chronic musculoskeletal pain is a common condition causing disability [62]. One example of chronic musculoskeletal pain is lateral epicondylalgia (LE), an overloading condition affecting the musculotendinous structures at the lateral epicondyle with high rates of persistent and recurrent pain [50]. Clinically, sensorimotor dysfunction is commonly reported in LE, manifesting as hyperalgesia around the lateral elbow, decreased grip force, and pain-related to wrist movement [6,8,14,21]. Recent studies have demonstrated increased overall excitability of motor cortex, reduced number of cortical peaks, and less intra-cortical inhibition and facilitation in LE compared with healthy subjects [8,48]. These data suggest cortical disinhibition and a shift towards greater cortical excitability in LE [8] but if these changes are causally linked to pain or to musculotendinous overloading is unclear [5,8]. Using experimental models of sustained pain may help to clarify the causal drive underpinning cortical excitability changes in LE.

Somatosensory evoked potentials (SEPs) and motor evoked potentials (MEPs) have been used to assess the neuroplastic consequences of pain since they are generated in diffuse areas of the sensorimotor cortex [4,15,18,44]. SEPs recorded over the centro-parietal and frontal cortices 20-60 ms after upper limb electrical stimulation are representative of the earliest relay and processing of afferent inputs in the primary sensory (S1) and premotor cortex (PMC), respectively [4,10,18,32]. Using transcranial magnetic stimulation (TMS), motor maps volume reflecting the overall excitability of the cortical representation of a muscle can be generated [64]. Combining these techniques allows investigation of several aspects of sensorimotor cortex functionality in response to pain [2,39].

Experimental pain models have been used to investigate mechanisms underlying sensorimotor excitability during muscle pain. Recently, models of sustained pain that mimic typical behaviour of musculoskeletal pain conditions have been developed to study the involvement of nociceptive stimulation in the transition to sustained pain [25,55]. An experimental pain model based on injection of nerve growth factor (NGF) has been used to induce several days of muscle hyperalgesia around the lateral epicondyle of the elbow [5]. Applying the NGF pain model, an increase in motor map volume was found [47], suggesting neuroplastic consequences in the motor cortex related to the hyperalgesic condition. Unaccustomed eccentric exercise-inducing delayed onset muscle soreness (DOMS) in the wrist extensor muscle is another pain model that reduced range of movement and muscle power in the affected muscle group [29,53], similar to what is observed in patients with lateral epicondylalgia [6,21,54]. Interestingly, the DOMS model is accompanied by increased somatosensory cortex excitability and reduced corticomotor excitability of the affected muscle [31].

The first aim of the present study was to investigate whether combined injection of NGF and DOMS provokes greater muscle soreness and disability, increased hyperalgesia, and reduction of maximal grip force compared with intramuscular injections of NGF alone. The second aim was to investigate the sensorimotor cortical neuroplastic consequences of muscle soreness induced by DOMS in a muscle pre-sensitized by intramuscular injection of NGF.

METHODS

Subjects

Twenty-four healthy right-handed subjects (14 females) participated in the study, recruited through online advertising and flyers posted at Aalborg University. The subjects were randomly

divided into two groups: NGF group (7 females) and NGF+DOMS group (7 females). The age, height, and weight (mean \pm SD) for the NGF and NGF+DOMS groups were, respectively: 25.1 \pm 1.6 years and 26.7 \pm 1.2 years; 172.3 \pm 2.4 cm and 170.1 \pm 3.3 cm; and 73.3 \pm 4.3 kg and 68.6 \pm 4.8 kg. All subjects had no prolonged conditions of upper and lower limb pain, spine pain, neurological or other major medical disorder. The average duration of high/moderate physical activity per weeks (mean \pm standard deviation) for NGF+DOMS and NGF group were 2.5 \pm 1.6 h and 2.3 \pm 1.7 h, respectively. A transcranial magnetic stimulation (TMS) safety screen was completed before starting experimental procedures [41]. The study was performed according with the Helsinki declaration, approved by the local Ethics Committee (N-20160022), and was registered at ClinicalTrials.gov (NCT03354624). Written informed consent was obtained prior to study commencement.

Study protocol

The study comprised 4 sessions over 6 days. On Day-0, Day-4 and Day-6, data were collected in the following sequence: 1) Pain related questionnaires (muscle soreness, pain body chart, patient-rated tennis elbow evaluation), 2) neurophysiological testing (motor evoked potentials and somatosensory evoked potentials); 3) quantitative sensory and motor assessment (grip force, wrist extensor force, and pressure pain thresholds). On Day-2, the same assessments were performed except for neurophysiological testing since no corticomotor excitability changes were found affected in a previous study [47]. At the end of each session on Day-0, Day-2 and Day-4, both groups received an injection of NGF into the right extensor carpi radialis brevis (ECRB) muscle to induce muscle soreness. In addition, on Day-4, the NGF+DOMS group performed high intensity eccentric exercise of the right wrist extensor muscles before receiving the NGF injection.

NGF-induced muscle soreness

Injections of NGF into the ECRB muscle were applied to induce muscle soreness along the radial side of the right forearm [5,47]. This experimental pain model has been previously developed to mimic features of lateral epicondylalgia [5]. Sterile solutions of recombinant human Beta-NGF were prepared by the pharmacy (Skanderborg Apotek, Denmark). After cleaning the skin with alcohol, NGF (5µg/0.5 mL) was injected into the muscle belly of ECRB under real-time ultrasound guidance (SonoSite M-Turbo, FUJIFILM SonoSite, USA) using a 1-mL syringe with a disposable needle (27G).

Eccentric exercise-induced delayed onset muscle soreness (DOMS)

High intensity eccentric-exercise of the wrist extensor muscles was applied to induce delayed onset muscle soreness [29,52] on Day-4 only in the NGF+DOMS group. Participants were seated, holding a weight in the right hand (max 25 kg), with the forearm pronated and positioned on an armrest. Eccentric contractions of the right hand were performed from a maximally extended wrist position to a maximally flexed wrist position with a duration of at least 4 s. Sets of five repetitions were separated by approximately 1-min rest period. The first set started with a weight corresponding to 90% of the maximal voluntary contraction (MVC) and was repeated until the participant was not able to control the eccentric contraction over 4 s. When reaching this point of failure, the load was progressively reduced in steps of 10% MVC ending at a load of 50% MVC in the final set. The experimenter gave verbal encouragement and lifted the weight following the eccentric exercise to prevent the participant from performing concentric actions when moving from the flexed to the extended wrist position.

Pain rating questionnaires and diary

The participants were requested to rate the intensity of muscle soreness at the beginning of each experimental session on a 7-point Likert scale where 0 represented a complete absence of pain/soreness; 1: a light pain/soreness in the muscle felt only when touched/a vague ache; 2: a moderate pain/soreness felt only when touched/a slight persistent ache; 3: a light muscle pain/soreness when lifting objects or carrying objects; 4: a light muscle pain/soreness, stiffness or weakness when moving the wrist or elbow without gripping an object; 5: a moderate muscle pain/soreness, stiffness or weakness when moving the wrist or elbow; and 6: a severe muscle pain/soreness, stiffness or weakness that limits my ability to move [5,52].

Participants were asked to draw the area of muscle soreness on a body chart. The areas of the body chart drawings were calculated in arbitrary units (a.u.) using a scanning program (ImageJ v 2.0.0-rc-55 , NIH, Bethesda, USA) [46].

The patient rated tennis elbow evaluation (PRTEE) questionnaire was used to assess average pain and disability of the right arm at the start of each session referring to the 24-hour period prior data collection. Scores for pain (sum of 5 items with maximum total score of 50) and disability (sum of 10 items, divided by 2, with a maximum score of 50) were combined to give a total score ranging from 0 (no pain and no functional impairment) to 100 (worst pain imaginable with significant functional impairment) [30]

Motor evoked potentials and cortical motor maps

The motor evoked potentials (MEPs) elicited in the ECRB muscle were recorded with surface disposable silver/silver chloride adhesive electrodes (Ambu Neuroline 720) placed parallel with the

muscle fibres. A reference electrode was mounted on the right olecranon. The electromyographic signals were band-pass filtered at 5 Hz-1 kHz, sampled at 2 kHz, and digitized by a 16-bit data-acquisition card (National Instruments, NI6122). With a swimming cap marked with a 1 × 1 cm stimulation grid and orientated to the vertex of the head, the MEP was evoked using a transcranial magnetic stimulator (Magstim 200², Magstim Co. Ltd, Dyfed, UK) with a focal figure-of-eight coil (D70² Coil, Magstim Co. Ltd, Dyfed, UK), while the muscle was at rest. The resting motor threshold (rMT) was defined at the point on the scalp at which the highest amplitude MEPs were evoked in the ECRB muscle (hotspot), and was defined as the intensity at which 5 out of 10 successive stimuli evoked a MEP with amplitude of at least 50 µV. The stimulus intensity was then set at 120% rMT. The motor cortical map representing the ECRB activation was recorded based on MEPs evoked every 6 s with a total of 5 stimuli at each site on the stimulation grid [47]. All grid sites were pseudo randomly stimulated from the hotspot until no MEP was recorded (defined as <50 µV peak-to-peak amplitude) in all five stimuli at all border sites [47]. If the average peak-to-peak amplitude of the 5 MEPs evoked at that site was greater than 50 µV, the site was considered 'active' [47]. The number of active map sites and map volume (sum of MEPs from active sites) were calculated offline. The centre of gravity (CoG) was defined as the amplitude-weighted centre of the map [64] and was calculated by $\frac{\sum MEP_i \cdot X_i}{\sum MEP_i}$; $\frac{\sum MEP_i \cdot Y_i}{\sum MEP_i}$; where MEP_i represents mean MEP amplitude at each site with the coordinates X_i, Y_i [59]. All transcranial magnetic stimulation (TMS) procedures applied in this study adhered to the TMS checklist for methodological quality [13]. For each session the average peak-to-peak MEP amplitude at individual sites across subjects were linearly interpolated to generate the MEP maps used for illustration of group effects.

Somatosensory evoked potentials

Subjects sat in an armchair in a quiet, semi-darkened room. The SEPs were obtained by non-painful electrical stimulation of the right radial nerve at the wrist (square wave pulses, stimulus intensity three times the perceptual threshold, with a frequency of 2 Hz, duration 1 ms). To specifically evaluate the frontal and centro-parietal near-field potentials, somatosensory evoked potentials (SEPs) were recorded on the contralateral hemisphere [45]. An electrode cap including 64 electrodes was used (g.GAMMA cap2, Schiedlberg, Austria) where the F3, F1, Fc3, Fc1, C3, C1, Cp3, Cp1, P3 and P1 scalp sites were used and referred to the contralateral earlobe [36]. An additional electro-oculogram electrode (Fp1) was recorded superior to the left eye to monitor eye-related movements. The ground electrode in the cap was placed half way between the eyebrows. Electrode impedances were kept below 5 k Ω . Electroencephalographic signals were amplified (50000x) and sampled at 2400 Hz (g.HIamp biosignal amplifier; g.tec-medical engineering GmbH, Schiedlberg, Austria). Two blocks of 500 stimuli were collected for all trials, filtered offline at 5-500 Hz and all traces were visually inspected for artefacts (blinks, eye movements, or contraction of scalp musculature) and any contaminated epochs were rejected before averaging. On average, about 20% of the trials in each session were rejected due to the presence of artefacts, which is acceptable according to previous studies [32,42]. The artefact-free waveforms were averaged and the peaks P14, N18, P22, N30, P45 and N60 in the frontal leads (F3, F1, Fc3, Fc1,) and P14, N20, P25, N33, P45 and N60 in the centro-parietal traces (C3, C1, Cp3, Cp1, P3, P1) were identified (Figure 1) [18,32,45], normalised to the pre-stimulation interval (subtracting the mean amplitude in the interval from -100 ms to -20 ms before the electrical stimulation) and the amplitudes and latencies were extracted.

Assessment of contraction force

Participants were positioned with their right and left forearms in a neutral position and 45 degrees elbow flexion. Three MVC of right and left hands with strong verbal encouragement were recorded with a custom-made grip dynamometer, consisting of a strain gauge (CCT Transducers, Turin, Italy) interposed between two padded bars [5,47]. The force signal was sampled at 500 Hz and the maximal grip force among the three trials was extracted for analysis.

For the wrist extension force, participants were seated with their right forearm positioned in pronation and the hand formed a fist. The distal portion of the hand was in contact with a force transducer that recorded the force output during wrist extension, and the right elbow in contact with the table to avoid any trunk and shoulder compensations during the contraction. Isometric wrist extension force was recorded via a force sensor (MC3A 250, AMTI, Watertown, MA 02472-4800, USA) mounted above the hand. Three MVC were performed to record the force exerted during the wrist extension contractions [5,52]. The force signal was sampled at 500 Hz and the maximal wrist extension force among the three trials was used for analysis.

Pressure pain sensitivity

In accord with previous procedures [5,47], pressure pain thresholds (PPTs) were recorded using a handheld pressure algometer (1-cm² probe, Algometer type II, SOMEDIC Electronics, Solna, Sweden). PPTs were calculated as the mean of three trials where the pressure was increased at a rate of 30 kPa/s until the subject detected the PPT and pressed a button. The average PPT of the 3 measures at each site was used for analysis. PPTs were recorded bilaterally at the extensor carpi radialis (ECR) muscle and tibialis anterior (TA) muscle.

Statistics

All data are presented as mean and standard error of the mean (SEM). Statistical analysis was performed using SPSS Version 24 for Windows (Chicago, IL, USA). All data from all assessments were normality tested using visual inspection and the Shapiro-Wilk's test. Accordingly, Likert scores, PRTEE and Body chart data were analysed by a mixed model analysis of variance (ANOVA) with *Group* (NGF and NGF+DOMS) as the between group factor and *Days* (Day-2, Day-4 and Day-6) as the within subject factor. The PPTs and maximal force levels were analysed by a mixed model ANOVA with *Group* as the between group factor and *Days* (Day-0, Day-2, Day-4 and Day-6) as the within subject factor. For the neurophysiological data, a mixed-model ANOVA with *Group* as the between group factor and *Days* (Day-0, Day-4 and Day-6) as the within subject factor were performed on rMT, MEPs at the hot spot, number of Active Sites, MEP map volume, latitude CoG, longitude CoG, and amplitude and latency of SEPs. Where appropriate, post-hoc analyses were performed using Bonferroni multiple comparison tests. Statistical significance was set at $P < 0.05$, however to compensate for the use of multiple ANOVAs in the analysis of EEG data (10 recording sites) the P-value from the ANOVAs was Bonferroni corrected to $P < 0.005$ (i.e. $0.05/10$) for accepting significance.

RESULTS

NGF induced muscle soreness was worsened by the addition of DOMS

A Day*Group interaction was found for Likert scores of muscle soreness (ANOVA: $F_{2,44} = 4.43$; $P = 0.02$). Post-hoc tests showed an increase in Likert scores from Day-2 to Day-4 in both groups and from Day-4 to Day-6 in the NGF+DOMS group ($P < 0.02$). Post-hoc tests revealed higher Likert scores (indicating greater muscle soreness) in the NGF+DOMS group compared with the NGF

group at Day 6 ($P = 0.01$; Table 1). A Day*Group interaction was also found for the PRTEE (ANOVA: $F_{1.5,33.6} = 4.84$; $P = 0.02$). Post-hoc tests showed an increase in the PRTEE score from Day-2 to Day-4 in both groups and from Day-4 to Day-6 in the NGF+DOMS group ($P < 0.01$; Table 1). Post-hoc tests between groups were not significant at Day-6 ($P = 0.12$). Muscle soreness was perceived mainly along the radial side of the forearm in both groups (Figure 2). The ANOVA revealed a Day*Group interaction in the body chart size ($F_{2,44} = 3.55$; $p = 0.04$). Post-hoc tests showed an increase in the body chart area from Day-2 to Day-4 in the both groups and from Day-4 to Day-6 in the NGF+DOMS group ($P < 0.03$). Post-hoc tests between groups revealed a tendency for larger areas in the NGF+DOMS group compared with the NGF group at Day-6 ($P = 0.055$; Table 1).

NGF increased pressure pain sensitivity, but this did not worsen with the addition of DOMS

The ANOVA of PPTs measured over the right ECRB muscle revealed a main effect of Day ($F_{3,66} = 47.48$; $P < 0.01$; Table 1). Compared with Day-0, the PPT over the right ECRB muscle was reduced at Day-2, Day-4, and Day-6 ($P < 0.01$). No difference in the PPT was found over the left ECR muscle (ANOVA interaction: $F_{3,66} = 0.69$; $P = 0.56$), right TA muscle (ANOVA interaction: $F_{3,66} = 0.24$; $P = 0.82$) or left TA muscle (ANOVA interaction: $F_{3,66} = 0.61$; $P = 0.66$; Table 1) in response to NGF+DOMS or NGF alone.

NGF injection increased corticomotor excitability which was subsequently decreased by the addition of DOMS

A Day*Group interaction was found for map volume (ANOVA: $F_{2,44} = 4.82$; $P = 0.01$; Figure 3; Table 2). Post-hoc tests showed a progressive increase in map volume from Day-0 to Day-4 in both groups ($P < 0.01$). At Day-6, only the NGF+DOMS group demonstrated a reduction in map volume

compared with Day-4 ($P = 0.01$). Compared with Day-0, only the NGF group showed an increase in map volume at Day-6 ($P < 0.01$). Post-hoc test between groups revealed a tendency for larger MEP volume in the NGF group at Day-6 compared with the NGF+DOMS group ($P=0.07$).

Main effect of Days was found in the number of Active Sites (ANOVA: $F_{2,44} = 20.3$; $P < 0.01$) and MEP amplitudes at the hot spot (ANOVA: $F_{2,44} = 6.69$; $P < 0.01$). Compared with Day-0, the number of Active Sites and MEP amplitudes on the hot spot were increased at Day-4 ($P < 0.01$) and at Day-6 ($P < 0.02$).

No difference was found in rMT (ANOVA interaction: $F_{2,44} = 0.70$; $P = 0.50$), Longitude CoG (ANOVA interaction: $F_{2,44} = 0.84$; $P = 0.44$) or Latitude CoG (ANOVA interaction $F_{2,44} = 2.89$; $P = 0.07$) between Groups and Days.

NGF-injections provoked reduced frontal N30 amplitude and increased centro-parietal N33-P45 amplitude

Figure 4 shows the 10 recording electrodes located in the contralateral hemisphere to the right radial nerve stimulation. First, a widely distributed positive far-field P14 potential was detected in all recording electrodes [18,32] (Figure 4, F1 and P1), followed by the frontal far-field N18 potential (Figure 4, Fc1). The near-field N20 potential (Figure 4, P1), representing the earliest cortical response, was identified in the centro-parietal region, followed by the P25 positivity and N33 negativity [4,18] (Allison et al., 1989; Fuji et al., 1994) (Figure 4, Cp1 and C1). In contrast to the centro-parietal region, the frontal near-field P22 positivity was followed by a large frontal N30 negativity [10,18] (Figure 4, Fc1 and F1). Finally, a widely distributed P45 potential could be recognized in all recording sites, followed by a diffuse N60 potential [18] (Figure 4, C1).

Significant effects of Day were found for the frontal N30-peak amplitude in F3, F1, Fc3 and Fc1 (Table 3; ANOVA: $F_{2,44} > 8.58$; $P < 0.002$). The frontal N30-peak amplitude decreased at Day-4 and this reduction persisted at Day-6 compared with Day-0 in both groups ($P < 0.01$). See Table, Supplemental Digital Content 1 illustrating the N30-peak amplitude of all frontal recording sites (available at <http://links.lww.com/PAIN/A635>).

Significant effects of Day were found for the centro-parietal N33-peak amplitude in C3, C1, Cp3, Cp1, P3 and P1 (Table 3; ANOVA: $F_{2,44} > 6.16$; $P < 0.004$). The centro-parietal N33-peak amplitude decreased at Day-4 and this reduction persisted at Day-6 compared with Day-0 in both groups ($P < 0.01$). See Table, Supplemental Digital Content 2, illustrating the N33-peak amplitude of all centro-parietal recording sites (available at <http://links.lww.com/PAIN/A635>).

Finally, significant effects of Day were also found for the P45-peak amplitude in frontal and centro-parietal recording sites (Table 3; ANOVA $F_{2,44} > 10.84$; $P < 0.001$). The peak amplitude of P45 increased at Day-4 and this increase persisted at Day-6 compared with Day-0 in both groups ($P < 0.01$). See Table, Supplemental Digital Content 3 (available at <http://links.lww.com/PAIN/A635>), illustrating the P45-peak amplitude of all recording sites. For all recording sites, the peak amplitude of P14 (ANOVA interaction: $F_{2,44} < 2.50$, $P > 0.09$), N18/N20 (ANOVA interaction: $F_{2,44} < 1.85$, $P > 0.16$), P22/P25 (ANOVA interaction: $F_{2,44} < 3.65$, $P > 0.03$) and N60 (ANOVA interaction: $F_{2,44} < 3.72$, $P > 0.03$) were not altered between Groups and Days (Table 3). There were no latency changes for any of the peaks under investigation (data not presented).

DOMS provoked a decrease in maximal contraction force

The ANOVA of the right grip force revealed a Day*Group interaction ($F_{2,2,48.4} = 5.87$; $P < 0.01$; Table 1). Post-hoc tests revealed that maximal right grip force was decreased at Day-6 compared with

Day-0, Day-2, and Day-4 ($P < 0.01$), but only in the NGF+DOMS group. No difference in grip force was found for the left hand (ANOVA interaction: $F_{3,66} = 0.45$; $p = 0.71$).

The ANOVA of the wrist extension force revealed a Day*Group interaction ($F_{3,66} = 18.03$; $P < 0.01$; Table 1). Post-hoc tests showed that only the NGF+DOMS group decreased in the maximal right wrist extension force at Day-6 compared with Day-0, Day-2, and Day-4 ($P < 0.01$). Post-hoc test between group revealed lower force in the NGF+DOMS group compared with the NGF group at Day-6 ($P = 0.02$).

DISCUSSION

The present findings provide important insight into the nature of sensorimotor adaptation during sustained muscle soreness. In fact, DOMS in a system already sensitized by NGF provides a unique opportunity to evaluate the sensorimotor adaptations in response to a clinically relevant, acute exacerbation of muscle pain. The combined experimental models of injection of NGF and DOMS evoked greater muscle soreness, functional disability, areas of pain, and less maximal force compared with intramuscular injection of NGF alone. In addition, the study investigated the neuroplastic sensory-motor consequences of muscle soreness induced by DOMS in a pre-sensitized muscle injected by NGF. These data demonstrate that the corticomotor depressive effect of DOMS obstructed the increased motor map volume induced by NGF, suggesting that the acute exacerbation of muscle pain may have different consequences for corticomotor function. Finally, these data showed reduction of the frontal N30 amplitude and an increase in the centroparietal N33-P45 amplitude in response to intramuscular injection of NGF into the ECRB muscle, but in contrast to motor map excitability, no somatosensory changes were found between groups.

DOMS increased the muscle soreness and disability induced by NGF and reduced maximal force

Chronic LE is a disabling pain condition, common in people who perform manual tasks with repeated, rapid movements of the wrist and forearm [16], that involves sensorimotor changes, including muscle hyperalgesia and a reduction in maximal grip force [6,14]. Whether these sensorimotor impairments are the cause or effect of sustained pain remains unclear, and intramuscular injections of NGF [5,33,47] and DOMS [20,51,52] have been used to help to clarify the causal drive for sensorimotor changes. Consistent with previous studies [5,47], two intramuscular injections of NGF into the ECRB muscle evoked muscle soreness along the radial side of the forearm, induced moderate muscle soreness (Likert scale: ~4, [47]) and disability in hand function (PRTEE: ~25, [47]), and an increase in sensitivity to mechanical pressure (PPT: ~100kPa, [47]). However, the decrease in maximal wrist extension and grip force was only substantial when eccentric exercise was applied to the tissues at Day-6 in the NGF+DOMS group. This is consistent with previous findings using DOMS as a means of generating muscle damage (20% reduction of max wrist extension force [29,52]). In fact, eccentric exercise involving the repetitive lengthening of muscle is known to cause damage to the ultrastructural components of muscle fibres [3]. Furthermore, muscle soreness induced by eccentric exercise develops 1 or 2 days after exercise, due to release of algescic substances from the damaged muscle, such as NGF, bradykinin and glial cell line-derived neurotrophic factor (GDNF) [34,35]. Therefore, a possible explanation for the increase in muscle soreness, hand function disability and areas of pain seen in the NGF+DOMS group may be the release of additional algescic substances from the damaged muscle. In contrast, the third injection of 5 µg of NGF into the muscle did not induce additional increase in muscle soreness probably caused by saturation of NGF-receptor in the area of the injections. These findings are in line with a previous report applying 3 injections of NGF in TA muscle where there

was no progressive increase of muscle soreness and decrease in the PPT 24 h after the second and three injections [25]. Collectively, the combined NGF and DOMS pain model used in the present study could be a suitable model of sustained lateral elbow pain similar to the key sensorimotor features of LE. Importantly, such experimental model of sustained pain cannot replicate important features of chronic LE such as long-term pain and functional limitation (>6 weeks), anxiety, fear of movement and fear of re-injury.

NGF induced soreness extended the ECRB motor map which subsequently was depressed by DOMS

Corticomotor excitability in patients affected by persistent musculoskeletal pain has been widely investigated [54,57,58] and interpreted as a sign of maladaptive neuroplasticity. When prolonged muscle soreness is induced by injection of NGF, increased map volume of the affected muscle has been described [47] consistent with the present findings on Day-4 and Day-6 in the NGF group. Based on a motor learning effect [37], the increase in motor map volume has been interpreted as an adaptive neuroplastic effect underpinning the search for a new strategy of movement [47]. This hypothesis is supported by previous studies showing expansion of motor map volume during the acquisition of new motor skills [38]. In contrast, when prolonged muscle soreness is induced by DOMS, reduction of motor map volume and reduction in maximal force have been described [31], probably caused by a neural protective mechanism to limit those movements involving damaged muscles [61] or an impairment in the peripheral excitation-contraction coupling process [63]. Similarly, the current study showed a reduction of the motor map volume when DOMS was applied in a pre-sensitized muscle, suggesting that the depressive corticomotor effects of muscle damage may interfere with the search for new strategy of movement. Interestingly, the induction of acute short-lasting muscle pain in a system already sensitized by NGF produced opposite effects

on corticomotor excitability [47] to those described when acute short-lasting muscle pain is induced in healthy individuals [40,56], probably by an increase in spinal excitability [47]. In contrast, H-reflexes after eccentric exercise were decreased immediately and 24 h after exercise, potentially explained by increased presynaptic inhibition of Ia afferents [61]. Therefore, these peripheral or spinal inhibitory effects provoked by muscle damage may explain attenuated corticomotor excitability found in the present study.

SEP adaptation after days of NGF-induced muscle soreness

Structural and functional reorganization of somatosensory cortical areas have been described in chronic musculoskeletal pain patients [19,22,49], suggesting that chronic pain is accompanied by cortical sensory reorganization. In addition, neuroplastic changes in the somatosensory cortex have been demonstrated in human pain models applying short-lasting acute pain [9]. However this is the first study to investigate the effect of prolonged muscle soreness induced by injection of NGF into a muscle. The main SEP components affected in the present study were frontal N30 and parietal N33-P45, suggesting that both frontal and parietal cortices were affected by muscle soreness. In addition, DOMS failed to show any statistical difference in the SEPs compared with the only injections of NGF.

Although the nature of frontal and parietal SEPs is unclear [4,60], cortical-surface recordings and transcortical recordings suggest that these potentials reflect different frontal and parietal cortical generators. Evidence from several studies show that sensory inputs reach pre-motor areas (premotor cortex (PMC) and supplementary motor area (SMA)) either after synapsing in S1 [26] or via parallel direct pathways from thalamic relays [32,45]. In particular, the frontal N30 component of somatosensory evoked potentials has been recognized to reflect some aspects of the sensory

information in motor planning and motor execution in PMC and SMA [10,11]. Although still debated, reduction of frontal N30 component has been described in several extrapyramidal syndromes, such Parkinson disease [43], Huntington's disease [1] or lesion of the prefrontal motor areas [24,32]. In addition this frontal component has also been associated with dopamine function since single doses of L-Dopa in Parkinson's patients increased the frontal N30 amplitude [12,27]. Therefore the N30 component has been related to the functionality of a complex cortico/subcortical loop linking ganglia, thalamus, supplementary and pre-motor areas [7]. The results of the current study showed for the first time that muscle soreness induced by NGF is able to decrease frontal cortical evoked potentials, suggesting that muscle soreness may interfere with some aspects of motor planning or motor execution.

The centro-parietal N33-P45 components of somatosensory evoked potentials were also affected in this study. Whereas centro-parietal SEP components elicited after to 60 ms originate in higher order somatosensory cortices, early SEPs components originate in the primary sensory cortex [4]. Studies in animal and human subjects converge to suggest a prominent role of this area in sensory discriminative aspects of both pain and touch perception [42, 28]. In addition, neuroimaging and neurophysiological studies indicate that the primary sensory cortex is involved in several chronic musculoskeletal pain [22,49] and experimental acute pain [42,53], suggesting that also muscle soreness induced by NGF may alter the somatosensory cortical excitability. However, changes of early SEPs, in particular P45 amplitude, can be also explained by changes in selective spatial attention [17,23]. Based on these previous studies, it is also possible that muscle soreness on the right forearm may provoke attention changes towards the stimulated territory. However, muscle soreness induced by DOMS and NGF is both conditions generally characterized by absence of muscle pain at rest. In addition, during the assessment of SEPs none of our

participants reported on-going muscle soreness, suggesting that spatial attention is unlikely the explanation of the changes in somatosensory cortex excitability.

Conclusion

This study shows for the first time that muscle soreness over several days induced by NGF was associated with neuroplastic adaptations of somatosensory evoked potentials generated by frontal and centro-parietal cortices, suggesting that muscle soreness may interfere in the sensorimotor integration and sensory discrimination of sensory inputs. In addition, inhibitory corticomotor effect of exercise-induced muscle soreness obstructed the increased motor map volume induced by NGF, suggesting that muscle damage may interfere with the search of new strategy of movement relevant for the pure soreness.

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FIGURE LEGENDS

Figure 1: Right radial nerve SEPs recorded by frontal electrodes (F3, F1, Fc3, Fc1) and centro-parietal electrodes (C3, C1, Cp3, Cp1, P3 and P1) scalp sites placed according to the 10-20 system. The N20 response is shown at the Cp1 site. P22 and P25 are indicated at the F1 and Cp1 sites and N30 and N33 are labelled at the F1 and P1 sites. P45 and N60 are indicated in the C1.

Figure 2: Superimposed body chart pain drawings (palmar and dorsal view of the right arm) showing distribution of muscle soreness at Day-2, Day-4, and Day-6 in NGF+DOMS (N = 12) and NGF (N = 12) group. Darker grey colours indicate a higher frequency of subjects reporting pain in that region.

Figure 3: Averaged (N = 24) peak-to-peak MEP amplitudes of the right ECR muscle interpolated across stimulation sites at Day-0 (before NGF) and Day-4 (after NGF injections). Averaged peak-to-peak MEP amplitudes of the right ECR muscle interpolated across stimulation sites at Day-6 in NGF+DOMS (N = 12) and NGF (N = 12) groups. The colour scale represents amplitude (from 0 to 800 μ V).

Figure 4: Grand average of SEPs from right radial nerve stimulation recorded by frontal electrodes (F3, F1, Fc3, Fc1) and centro-parietal electrodes (C3, C1, Cp3, Cp1, P3 and P1) scalp sites placed according to the 10-20 system. Averaged SEPs across both groups at Day-0 (blue line, N = 24) and Day-4 (red line, N = 24) and at Day-6 in the NGF group (black line, N = 12) and NGF+DOMS group (black dash line, N = 12). P14, presented over all the traces, is indicated in the F1 and P1 traces. The N18 and N20 responses are shown at the Fc1 and P1 traces, respectively. P22 and P25 are indicated at the Fc1 and Cp1 sites and N30 and N33 are labelled at the F1 and C1 sites. P45 and N60, presented over all the traces, are indicated in the C1.

	Group	Day-0	Day-2	Day-4	Day-6
Likert scores (0-6)	NGF	-	3.7 ± 0.3	4.2 ± 0.3 ^b	4.3 ± 0.2 ^{b,*}
	NGF+DOMS	-	3.5 ± 0.2	4.3 ± 0.3 ^b	4.9 ± 0.2 ^{b,c,*}
PRTEE (0-100)	NGF	-	17.0 ± 2.3	25.0 ± 3.6 ^b	24.3 ± 3.8 ^b
	NGF+DOMS	-	16.0 ± 1.9	24.4 ± 3.3 ^b	34.5 ± 5.1 ^{b,c}
Area of soreness (a.u.)	NGF	-	308.7 ± 44.6	392.5 ± 46.3 ^b	395.7 ± 29.1 ^b
	NGF+DOMS	-	290.1 ± 53.6	395.0 ± 52.9 ^b	555.7 ± 73.4 ^{b,c}
PPT left ECRB muscle (kPa)	NGF	191.3 ± 22.1	190.4 ± 23.7	179.6 ± 15.8	192.6 ± 17.6
	NGF+DOMS	212.3 ± 17.9	216.1 ± 23	213.2 ± 20.9	202.4 ± 18.7
PPT right ECRB muscle (kPa)	NGF	209.5 ± 24.6	103.2 ± 11.5 ^a	93.4 ± 8.9 ^a	92.3 ± 12.6 ^{a,b}
	NGF+DOMS	217.1 ± 20.3	129.7 ± 16.9 ^a	108.7 ± 12.2 ^a	100.2 ± 13.7 ^{a,b}
PPT left TA muscle(kPa)	NGF	450.4 ± 60.7	446.0 ± 62.8	427.7 ± 52.4	475.7 ± 60.1
	NGF+DOMS	470.2 ± 42.9	461.1 ± 40.6	473.2 ± 47.9	487.5 ± 47.0
PPT right TA muscle (kPa)	NGF	483.7 ± 65.4	478.9 ± 56.2	443.8 ± 47.2	484.2 ± 53
	NGF+DOMS	481.6 ± 38.0	479.6 ± 34.3	471.8 ± 43.5	491.4 ± 49.3
Right wrist Force (N)	NGF	152.0 ± 8.1	149.1 ± 8.4	150.1 ± 8.7	150.2 ± 7.8 [*]
	NGF+DOMS	147.1 ± 9.3 ^d	142.9 ± 9.0 ^d	141.9 ± 10.1 ^d	121.5 ± 9.0 [*]
Left grip force (kg)	NGF	30.4 ± 2.4	31.1 ± 2.4	29.4 ± 8.3	30.1 ± 2.6
	NGF+DOMS	29.5 ± 1.8	29.6 ± 1.9	28.7 ± 1.8	29.0 ± 1.9
Right grip force (kg)	NGF	33.5 ± 2.3	32.8 ± 2.4	31.6 ± 2.3	32.9 ± 2.4
	NGF+DOMS	32.7 ± 2.3 ^d	32.4 ± 2.0 ^d	30.7 ± 2.0 ^d	28.7 ± 2.0

Table 1: Mean (± SEM, N = 12) Likert scale, PRTEE, Area of soreness, pressure pain thresholds (PPTs) on extensor carpi radialis brevis (ECRB) and tibialis anterior (TA) muscles, maximal wrist extension force, and maximal grip force. Significantly different from Day-0 within the group (^a, P<0.05), from Day-2 within the group (^b, P<0.05), from Day-4 within the group (^c, P<0.05), from Day-6 within the group (^d, P<0.05) and between groups within the day (^{*}, P<0.05).

	Group	Day-0	Day-4	Day-6
rMT (%)	NGF	39.7 ± 2.0	39.4 ± 2.1	38.8 ± 1.8
	NGF+DOMS	37.1 ± 2.7	37.5 ± 2.5	37.3 ± 2.3
MEP amplitude (μV)	NGF	595.9 ± 123.8	795.7 ± 192.8 ^a	913.7 ± 166.2 ^a
	NGF+DOMS	561.9 ± 102.3	716.8 ± 107.3 ^a	616.4 ± 106.7 ^a
Map volume (μV)	NGF	3684.6 ± 432.0	5515.4 ± 733.0 ^a	5866.0 ± 748.9 ^a
	NGF+DOMS	3673.2 ± 461.7	5801.3 ± 659.6 ^a	4247.7 ± 458.9 ^b
Number of Active Sites	NGF	14.3 ± 0.8	18.6 ± 1.3 ^a	18.0 ± 1.1 ^a
	NGF+DOMS	13.8 ± 0.8	17.8 ± 0.9 ^a	16.3 ± 1.4 ^a
CoG latitude (cm)	NGF	5.9 ± 0.2	5.9 ± 0.1	5.7 ± 0.1
	NGF+DOMS	5.7 ± 0.2	5.7 ± 0.1	5.7 ± 0.2
CoG longitude (cm)	NGF	1.7 ± 0.1	1.8 ± 0.2	1.9 ± 0.1
	NGF+DOMS	1.7 ± 0.2	1.7 ± 0.1	1.8 ± 0.1

Table 2: Mean (± SEM, N = 12) parameters related with motor-evoked potentials. rMT: resting motor threshold. Significantly different from Day-0 within the group (^a, P<0.05) and from Day-4 within the group (^b, P<0.05).

SEP Component	Peak electrode	Group	Day-0	Day-4	Day-6
P14	F1	NGF	0.59 ± 0.09	0.55 ± 0.11	0.57 ± 0.13
		NGF+DOMS	0.66 ± 0.09	0.65 ± 0.10	0.48 ± 0.11
N18	Fc3	NGF	-0.19 ± 0.10	-0.17 ± 0.11	-0.05 ± 0.13
		NGF+DOMS	-0.26 ± 0.09	-0.33 ± 0.11	-0.12 ± 0.11
N20	Cp3	NGF	-0.89 ± 0.09	-1.06 ± 0.14	-0.96 ± 0.15
		NGF+DOMS	-0.83 ± 0.13	-0.98 ± 0.14	-0.91 ± 0.14
P22	Fc3	NGF	0.38 ± 0.12	0.36 ± 0.14	0.69 ± 0.14
		NGF+DOMS	0.49 ± 0.12	0.71 ± 0.12	0.79 ± 0.13
P25	Cp3	NGF	1.20 ± 0.27	1.24 ± 0.23	1.51 ± 0.30
		NGF+DOMS	1.51 ± 0.25	1.86 ± 0.31	1.77 ± 0.28
N30	F1	NGF	-1.78 ± 0.20	-1.16 ± 0.18 ^a	-1.16 ± 0.20 ^a
		NGF+DOMS	-2.05 ± 0.17	-1.49 ± 0.18 ^a	-1.62 ± 0.15 ^a
N33	Cp1	NGF	-0.62 ± 0.17	-0.18 ± 0.19 ^a	-0.07 ± 0.22 ^a
		NGF+DOMS	-0.42 ± 0.18	-0.05 ± 0.22 ^a	-0.01 ± 0.24 ^a
P45	Cp3	NGF	1.52 ± 0.26	2.10 ± 0.27 ^a	2.31 ± 0.33 ^a
		NGF+DOMS	1.70 ± 0.24	2.33 ± 0.29 ^a	2.24 ± 0.22 ^a
N60	Fc1	NGF	-1.24 ± 0.24	-1.18 ± 0.28	-1.02 ± 0.29
		NGF+DOMS	-1.63 ± 0.23	-1.37 ± 0.18	-1.60 ± 0.22

Table 3: Mean (± SEM, N = 12) for each component peak electrode. Significantly different from Day-0 within the group (^a, P<0.05).







